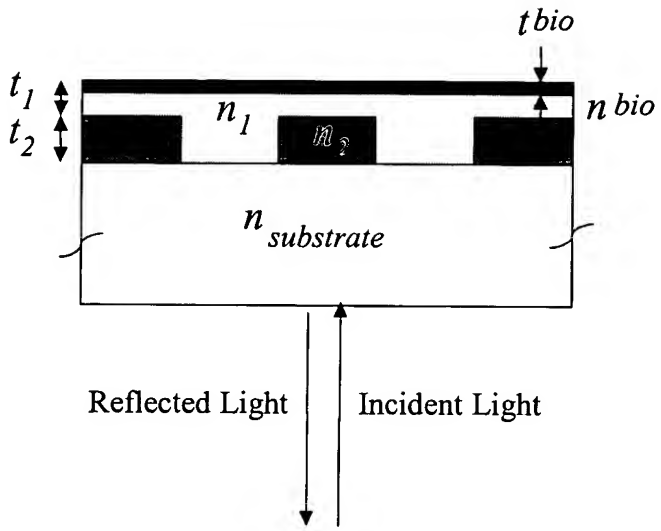

Figure 1A

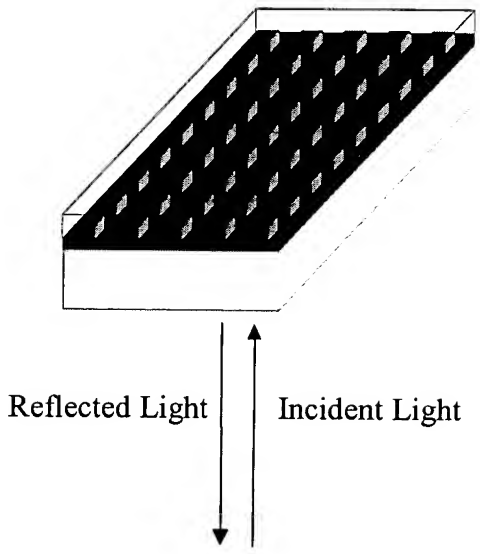

Figure 1B

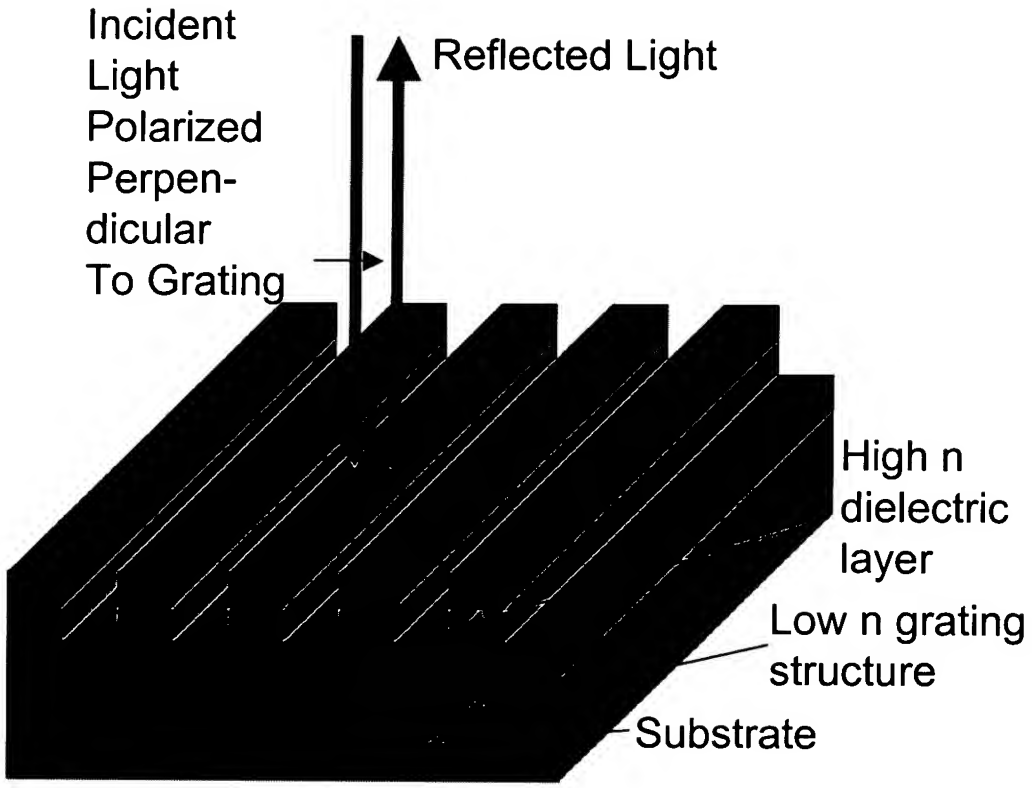
Incident
Light
Polarized
Perpen-
dicular
To Grating
Reflected Light
High n
dielectric
layer
Low n grating
structure
Substrate

Figure 2

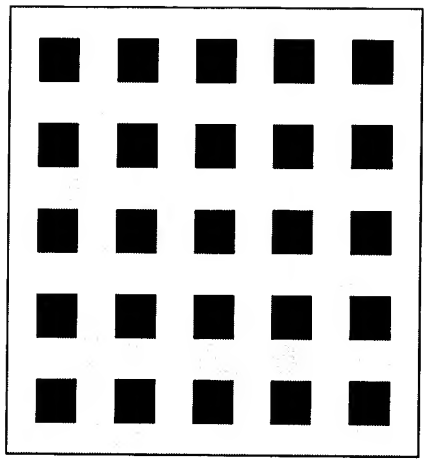

Figure 3A

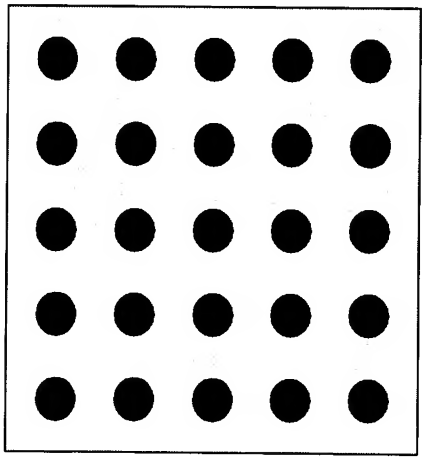

Figure 3B

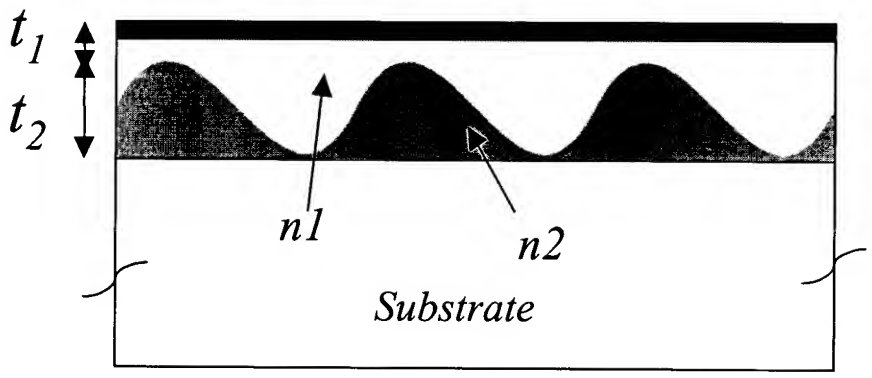

Figure 4

Concentric Circle Design

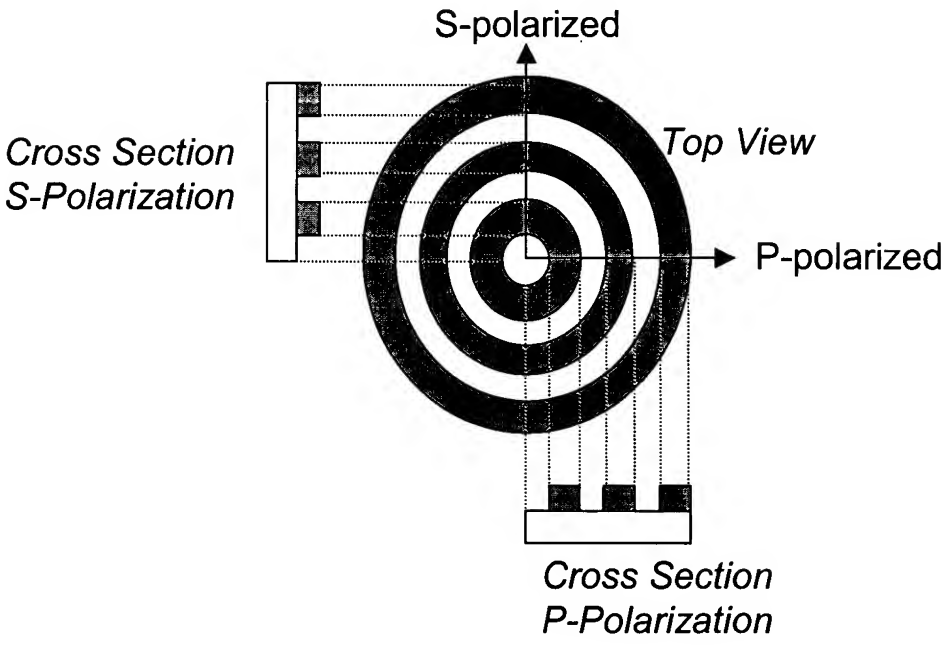

Figure 5

Hexagonal Grid Design

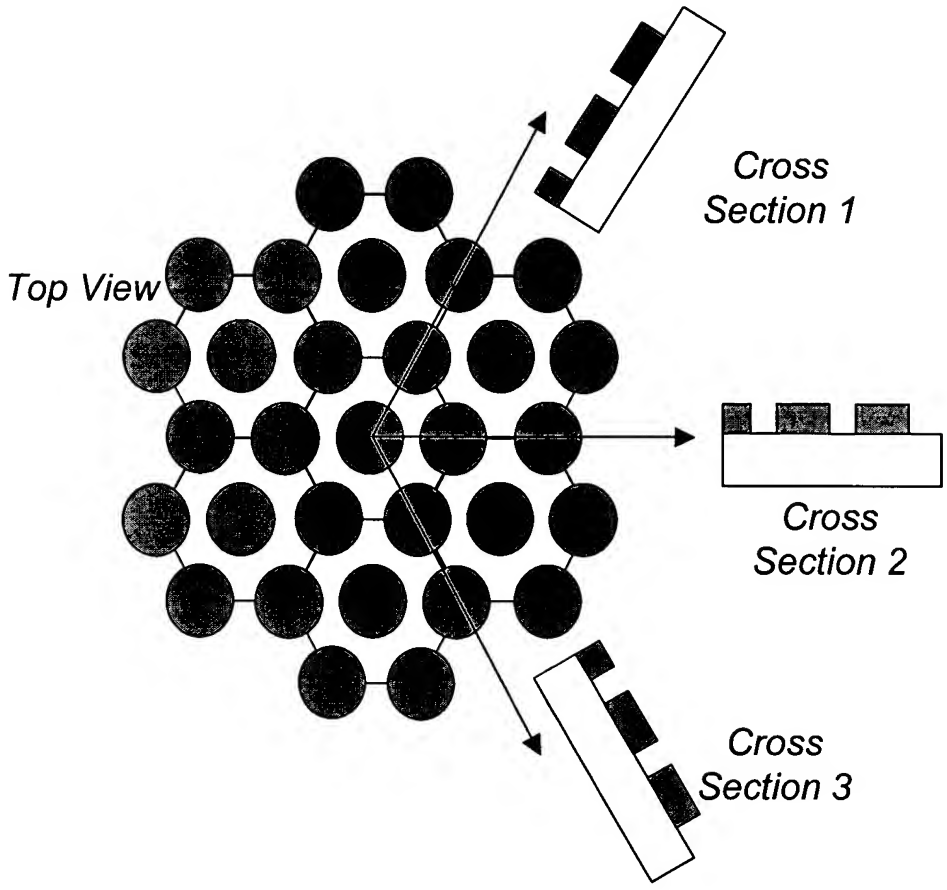

Figure 6

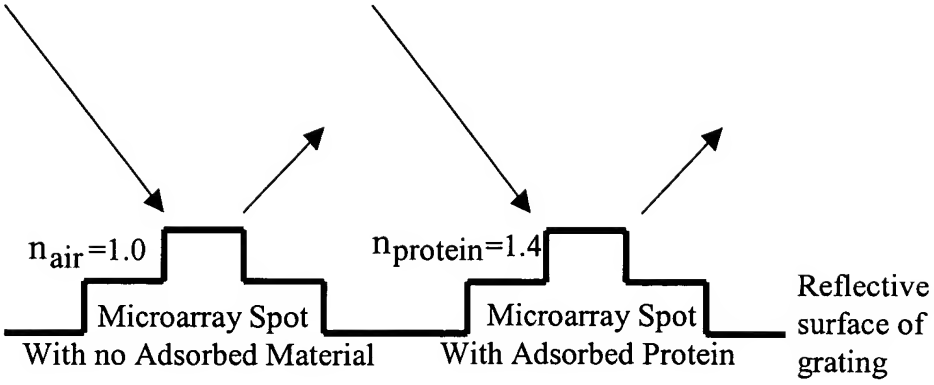
n_{air}=1.0
$n_{protein}$=1.4
Microarray Spot
With no Adsorbed Material
Microarray Spot
With Adsorbed Protein
Reflective
surface of
grating

Figure 7

Amine

- ➢Sulfo-succinimidyl-6-(biotinamido)hexanoate (Sulfo-NHS-LC-Biotin)
  - •Streptavidin / avidin then biotinylated molecule
- ➢N,N'-disuccinimidyl carbonate (DSC); • -NH_2, non-cleavable
- ➢Dimethyl pimelimidate (DMP); • -NH_2, non-cleavable
- ➢Dimethyl 3,3'-dithiobispropionimidate (DTBP); • -NH_2, cleavable
- ➢1-Ethyl-3-(3-Dimethylaminopropyl)carbodiimide Hydrochloride (EDC) and N-Hydroxysulfosuccinimide (Sulfo-NHS); • -COOH
- ➢Sulfo-succinimidyl 6-[a-methyl-a-(2-pyridyl-dithio)toluamido] hexanoate (Sulfo-LC-SMPT); • -SH, cleavable
- ➢N-(B-Maleimidopropyloxy)succinimide ester (BMPS)
  - • -SH_2, non-cleavable
- ➢Sulfo-succinimidyl 4-[N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC); • -SH, non-cleavable

Aldehyde

- ➢ Directly with aldehyde or first amino then aldehyde
  - • -NH_2

Ni(II)

- ➢Using Nitrilotriacetic acid (NTA) group, which forms a chelate with Ni(II)
  - • His-tagged molecules

Figure 8

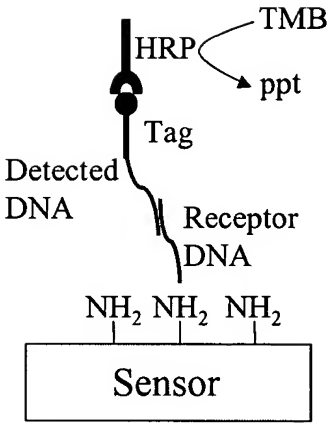

Figure 9A

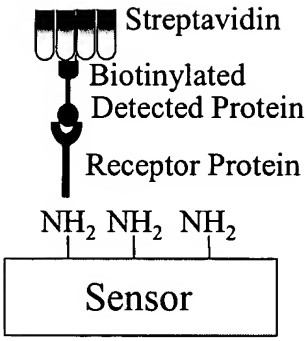

Figure 9B

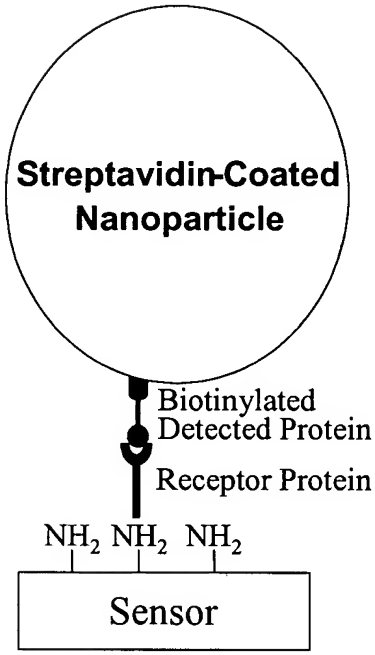

Figure 9C

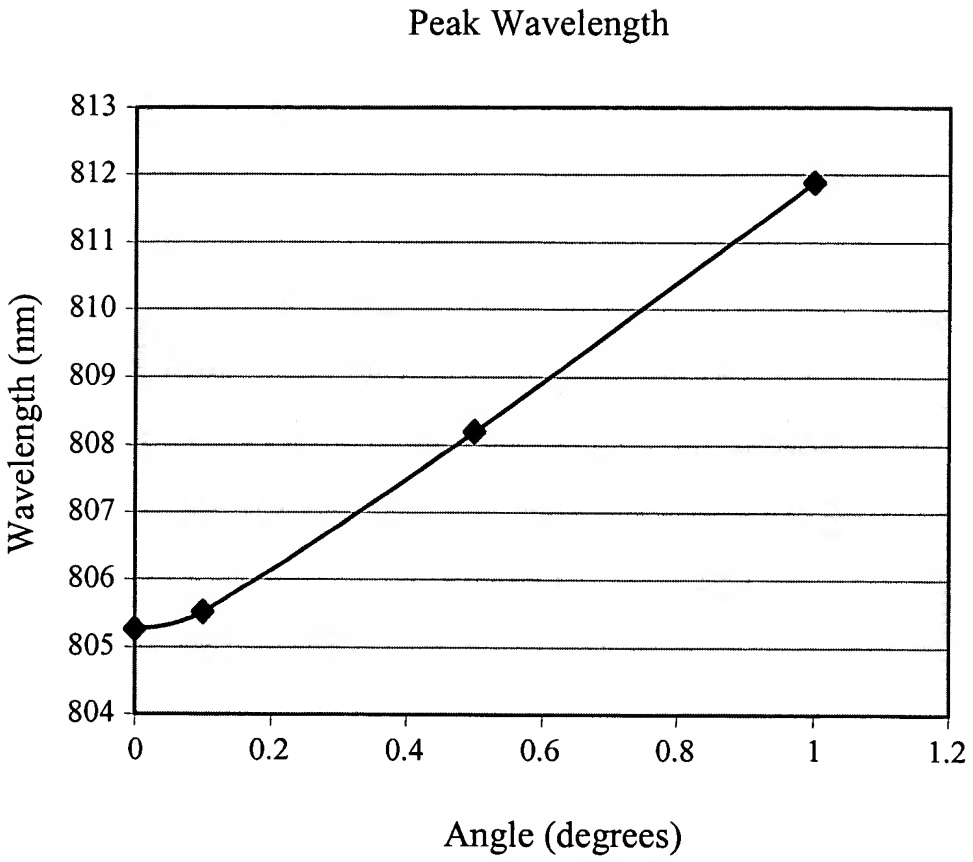
Peak Wavelength
Wavelength (nm)
813
812
811
810
809
808
807
806
805
804
0
0.2
0.4
0.6
0.8
1
1.2
Angle (degrees)

Figure 10

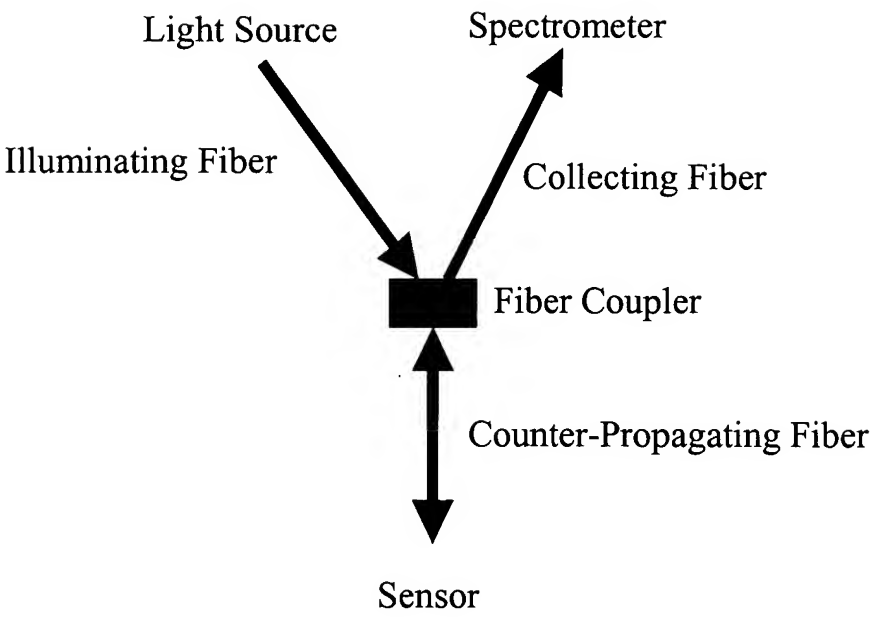
Light Source
Spectrometer
Illuminating Fiber
Collecting Fiber
Fiber Coupler
Counter-Propagating Fiber
Sensor

Figure 11

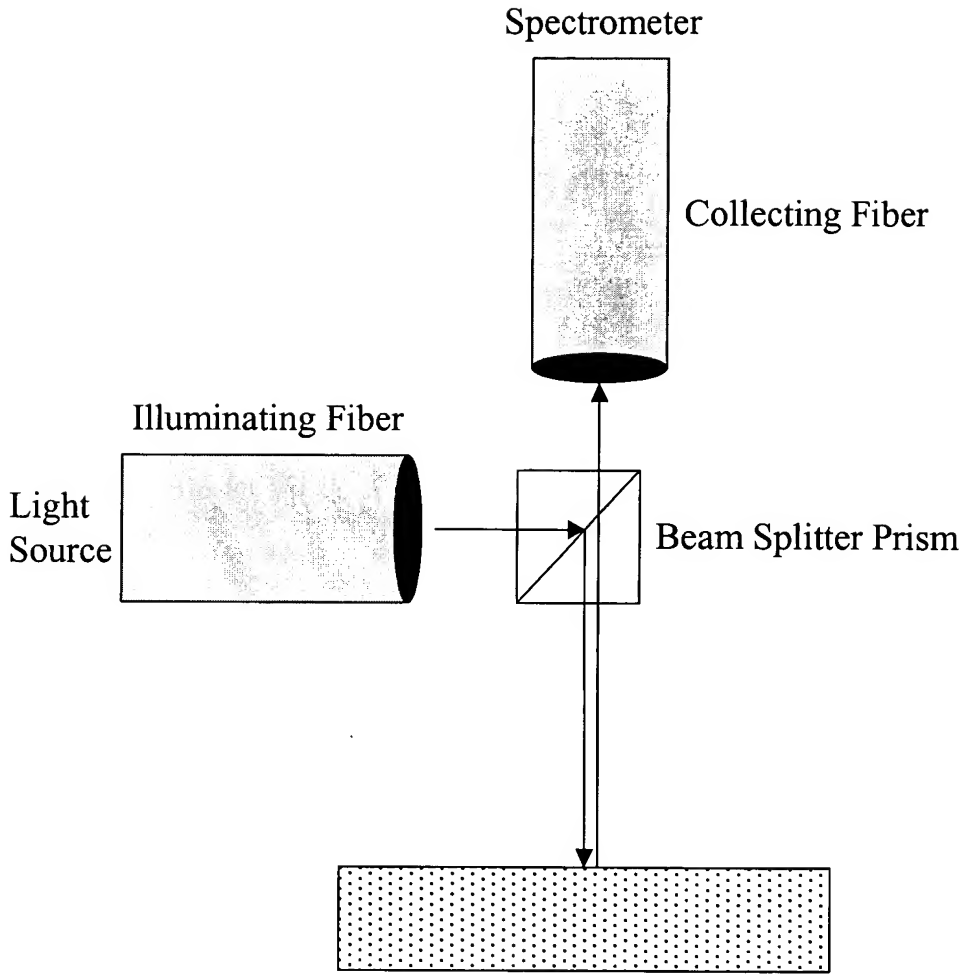
Spectrometer
Collecting Fiber
Illuminating Fiber
Light
Source
Beam Splitter Prism

Figure 12

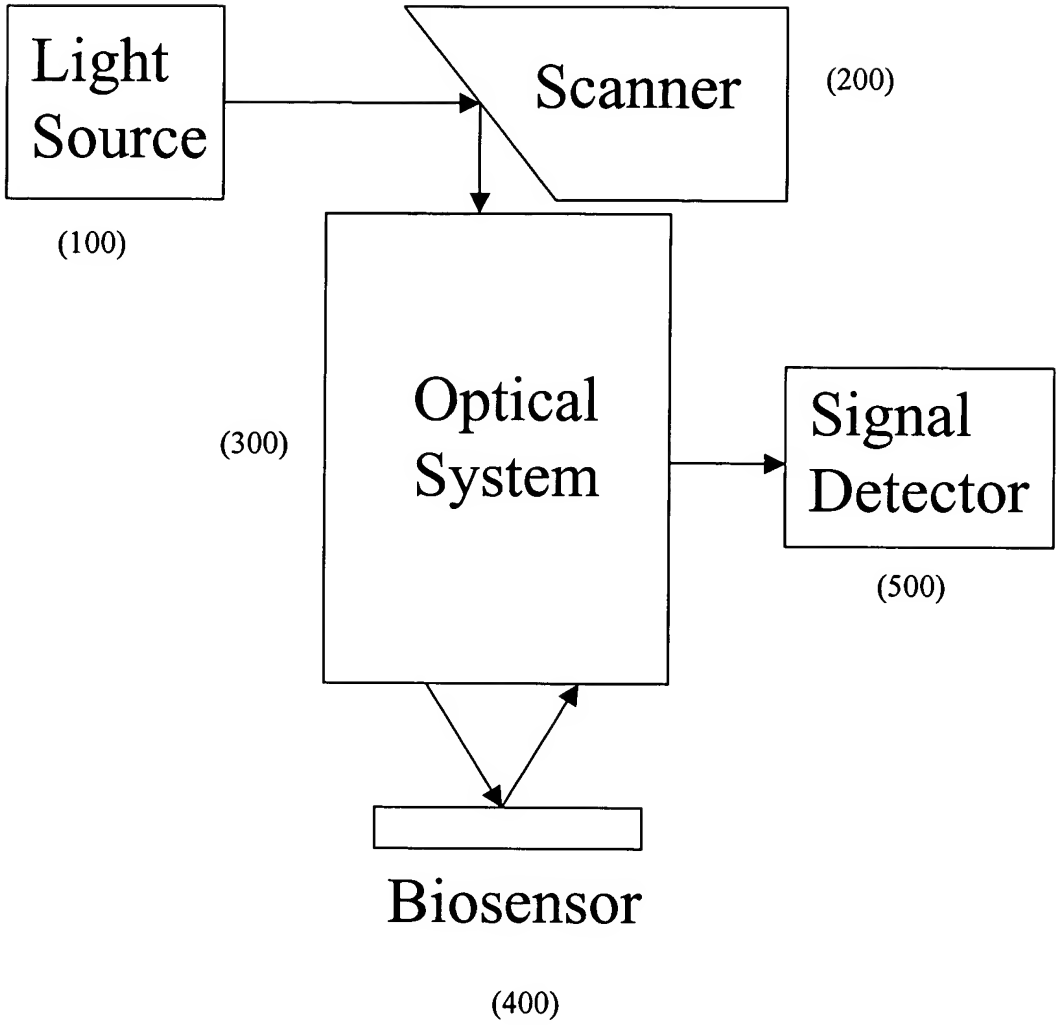
Light Source
(100)
Scanner
(200)
Optical System
(300)
Signal Detector
(500)
Biosensor
(400)

Figure 13

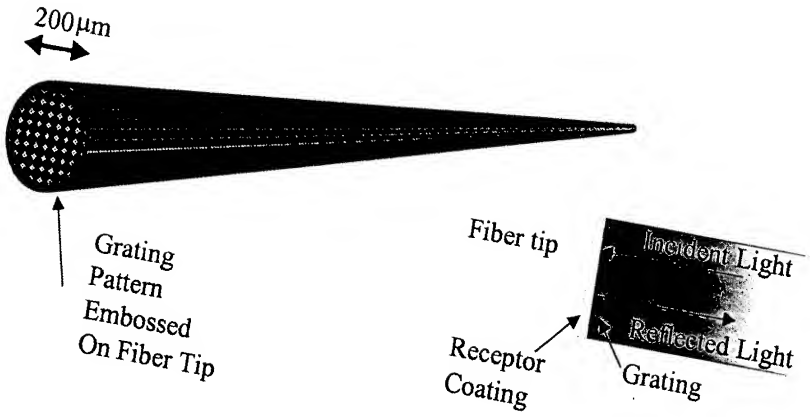
200μm
Grating
Pattern
Embossed
On Fiber Tip
Fiber tip
Incident Light
Reflected Light
Receptor
Coating
Grating

Figure 14

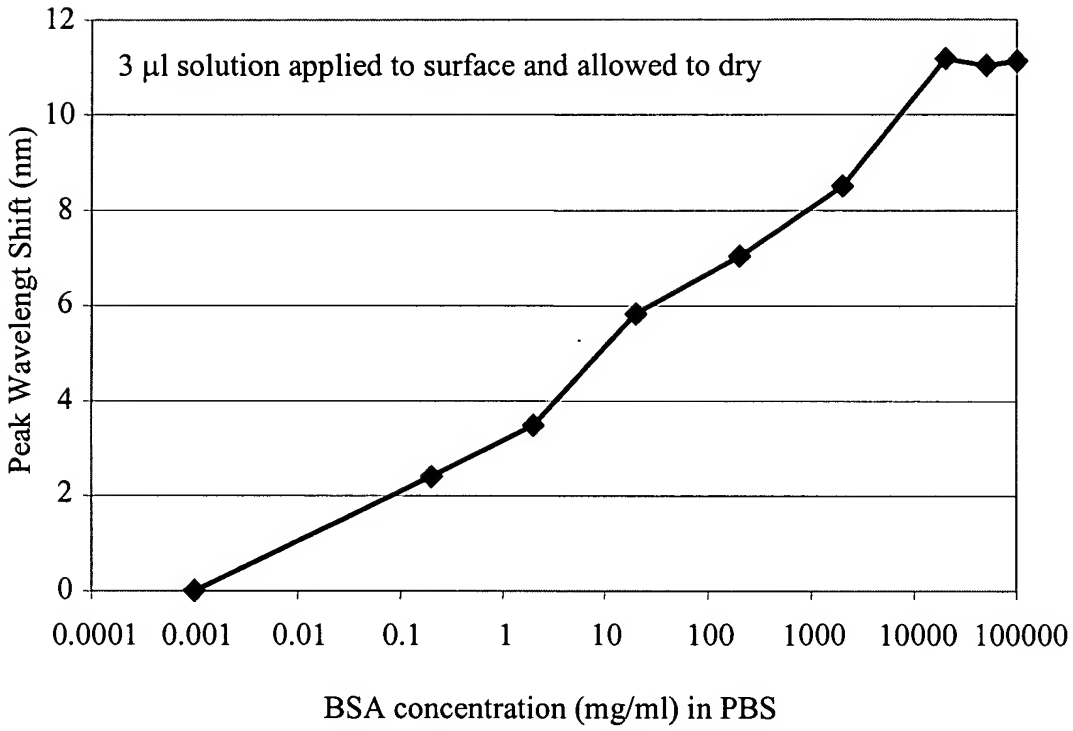
3 µl solution applied to surface and allowed to dry
Peak Wavelengt Shift (nm)
12
10
8
6
4
2
0
0.0001
0.001
0.01
0.1
1
10
100
1000
10000
100000
BSA concentration (mg/ml) in PBS

Figure 15

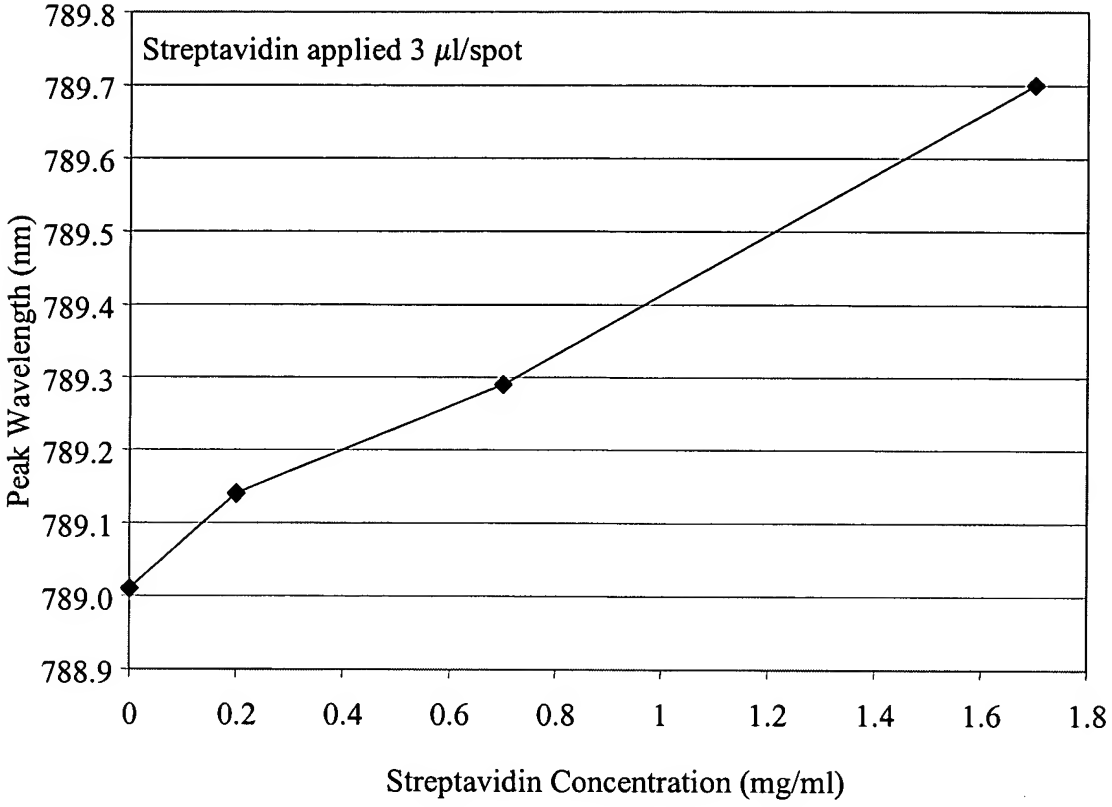

Figure 16A

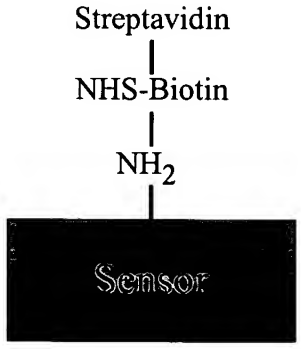

Figure 16B

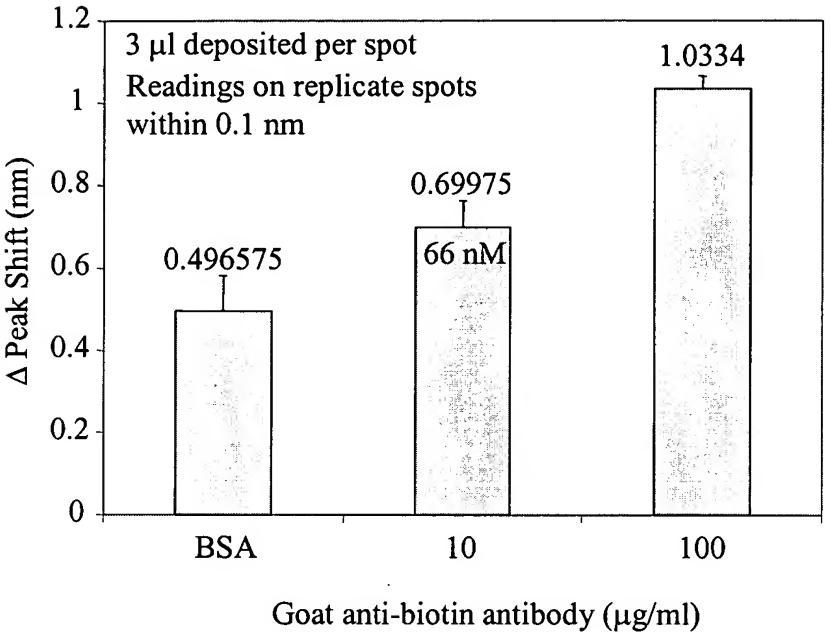

Figure 17A

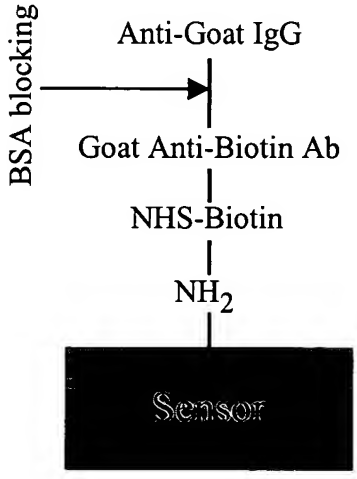

Figure 17B

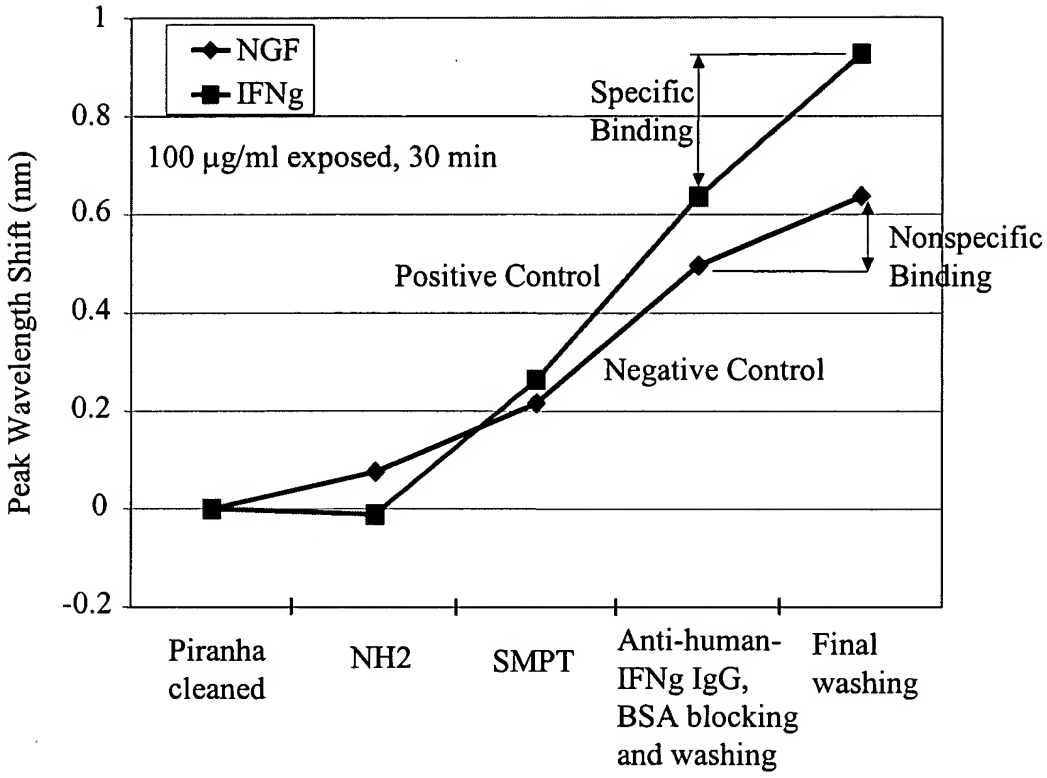

Figure 18A

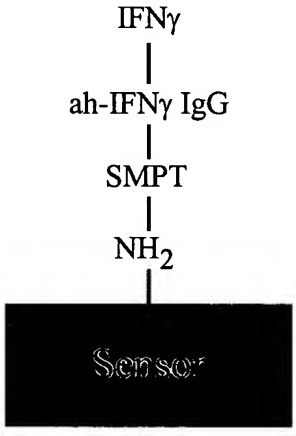

Figure 18B

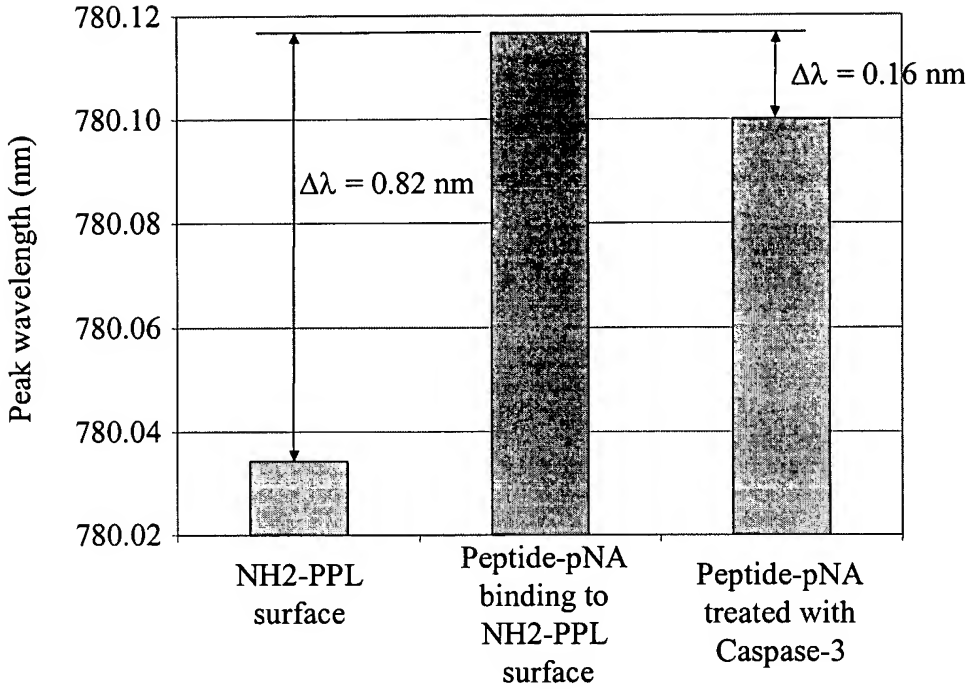

Figure 19A

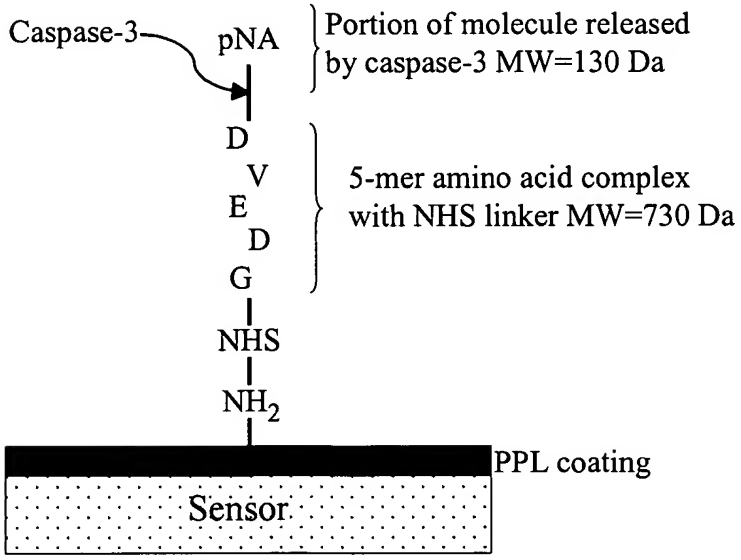

Figure 19B

Measured shifting of the resonant wavelength caused by the binding of various biomolecular layers.

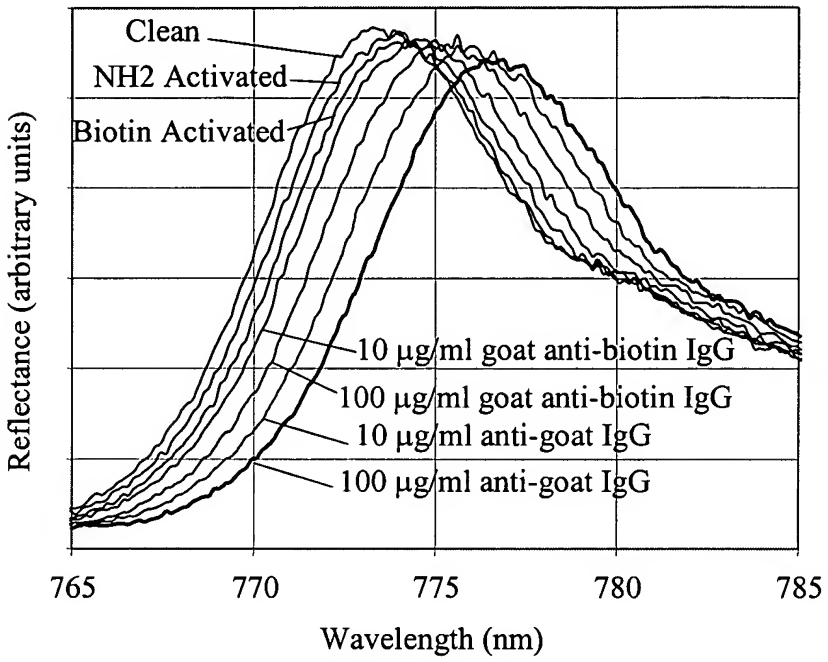

Figure 20A

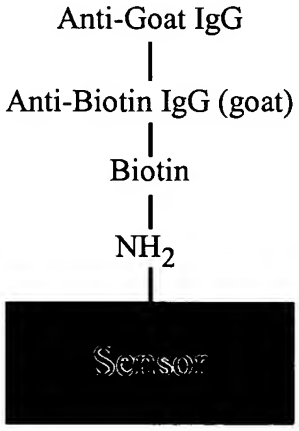

Figure 20B

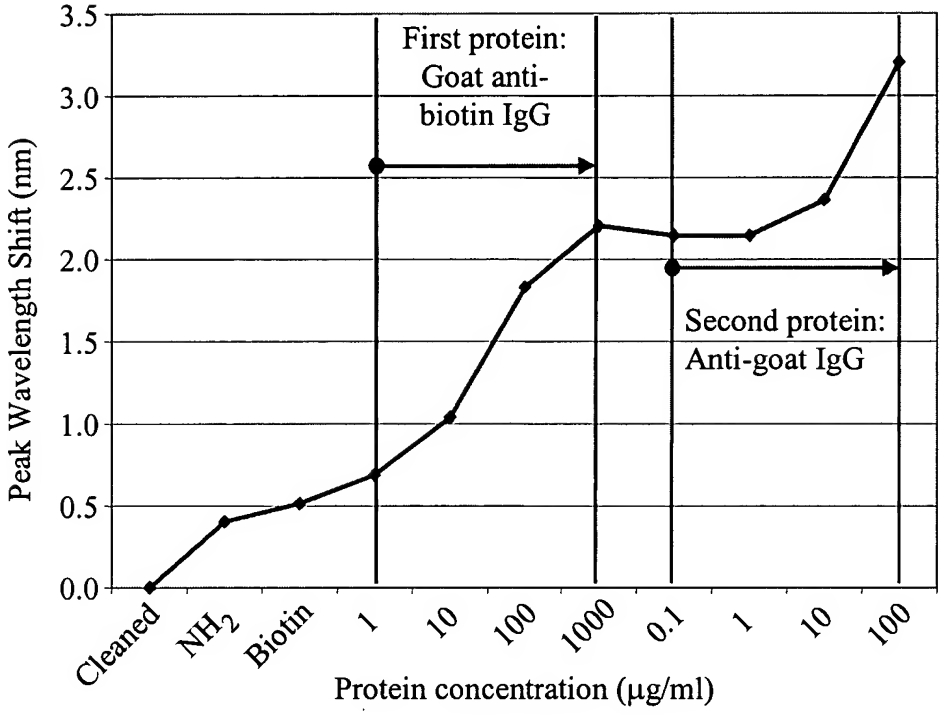

Figure 21A

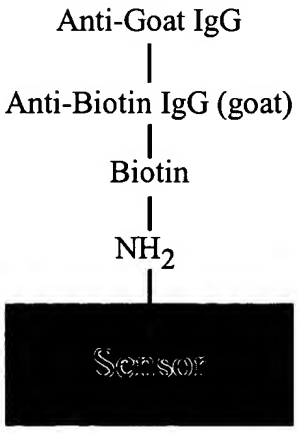

Figure 21B

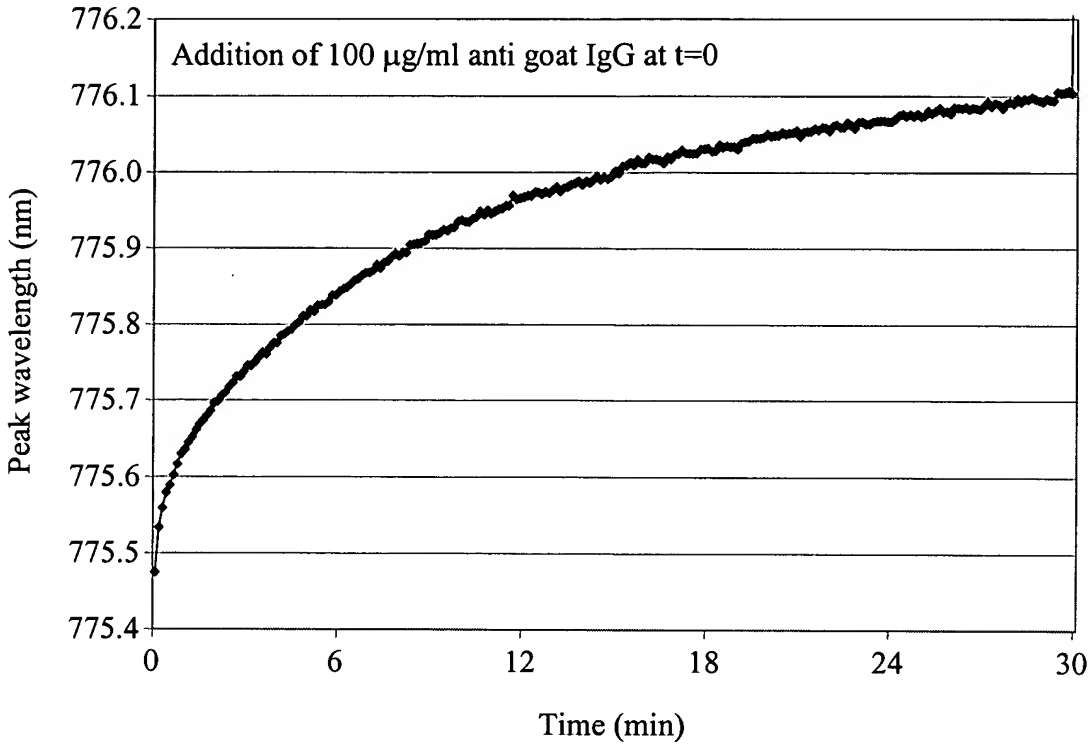

Figure 22A

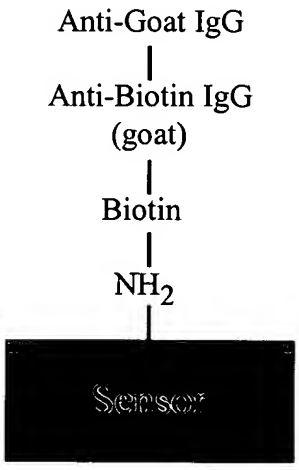

Figure 22B

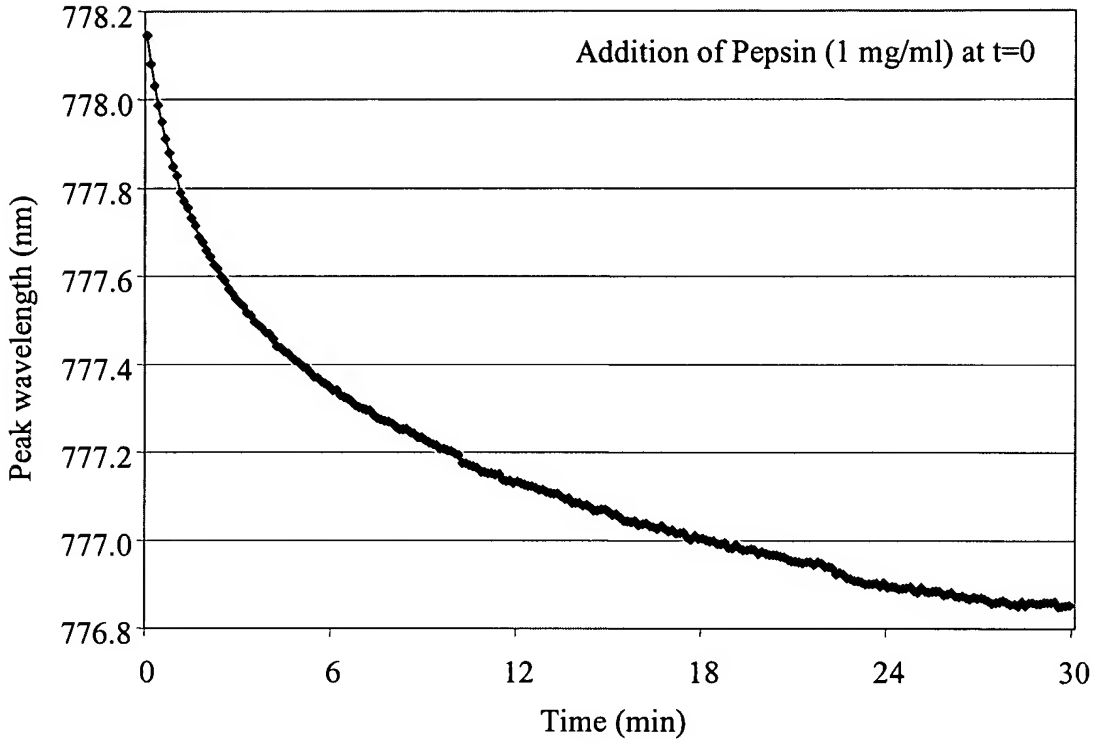

Figure 23A

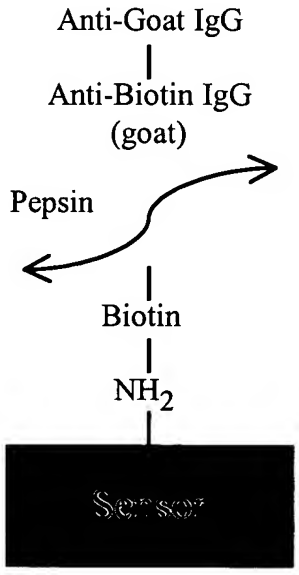

Figure 23B

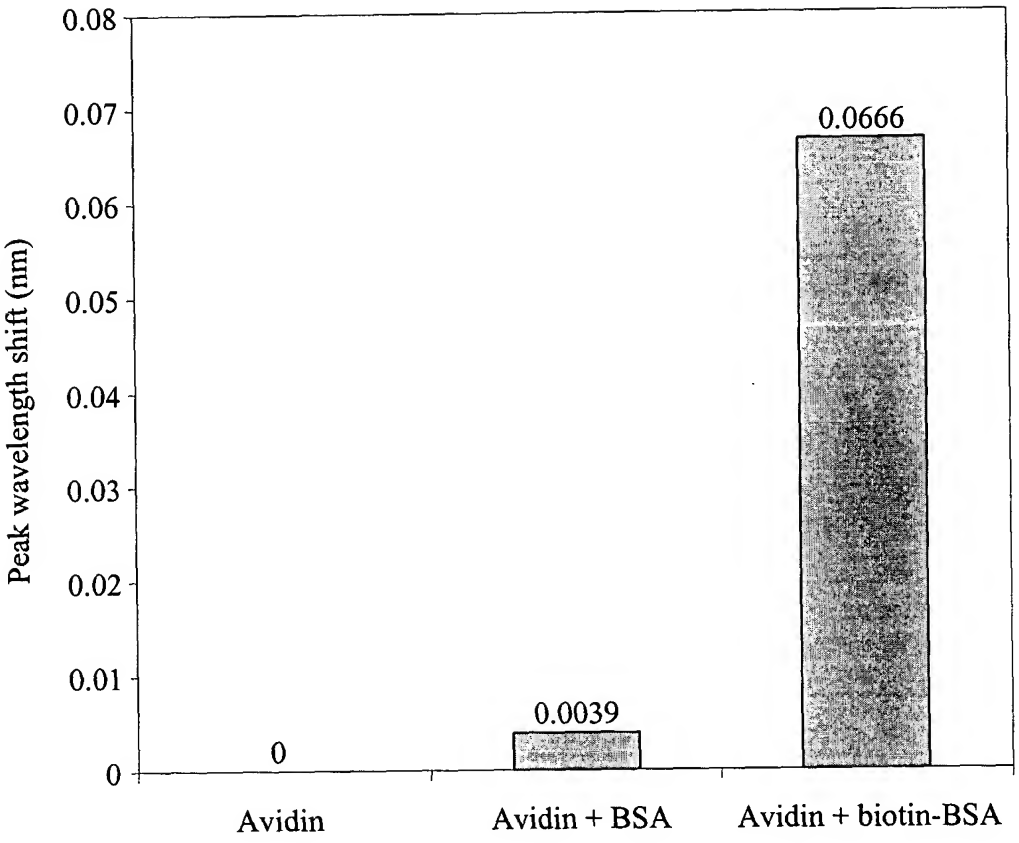
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Peak wavelength shift (nm)
0
0.0039
0.0666
Avidin
Avidin + BSA
Avidin + biotin-BSA

Figure 24

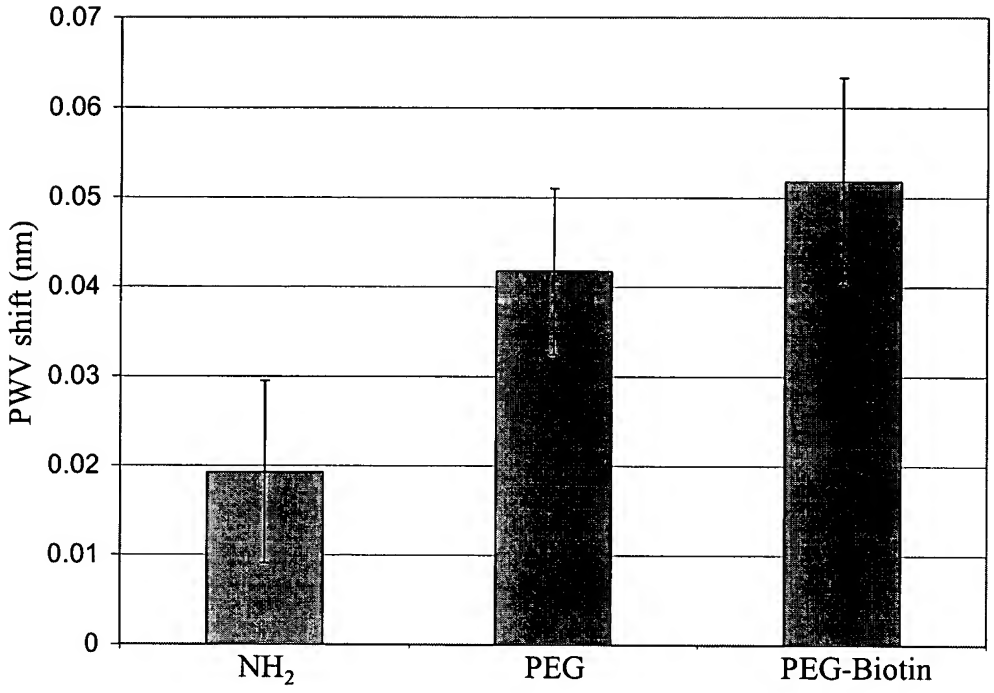

Figure 25

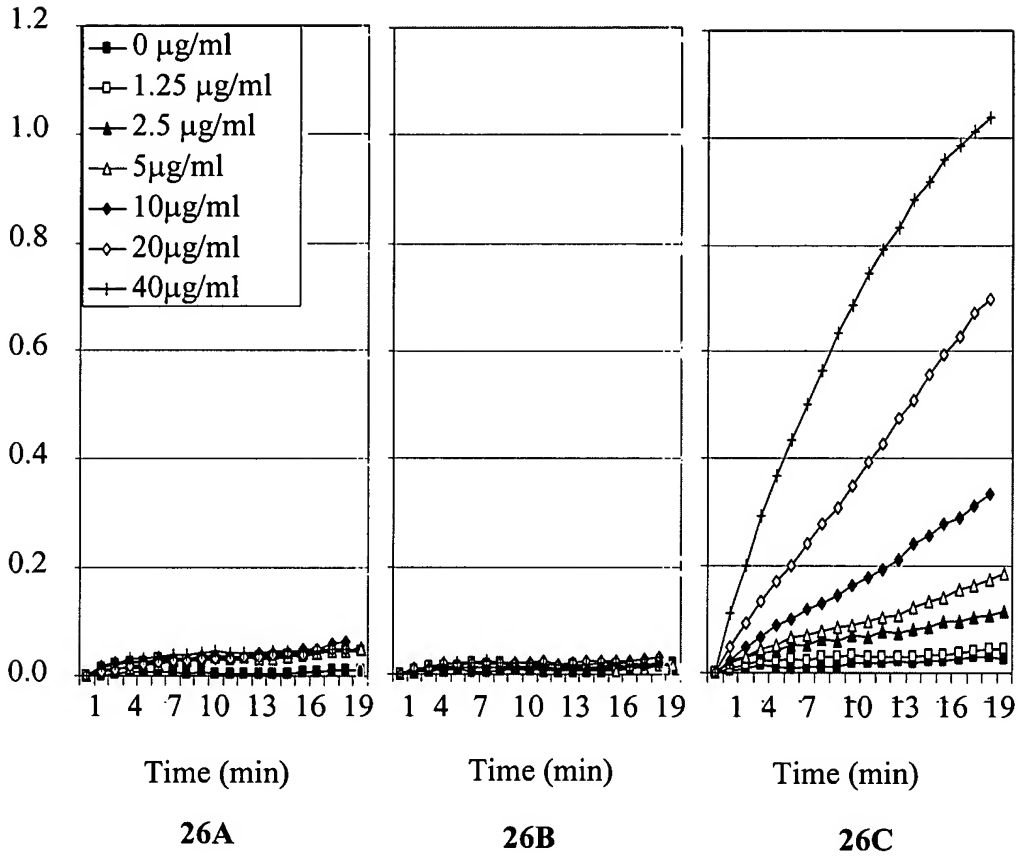
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 µg/ml
1.25 µg/ml
2.5 µg/ml
5µg/ml
10µg/ml
20µg/ml
40µg/ml
1 4 7 10 13 16 19
Time (min)
26A
26B
26C

Figure 26

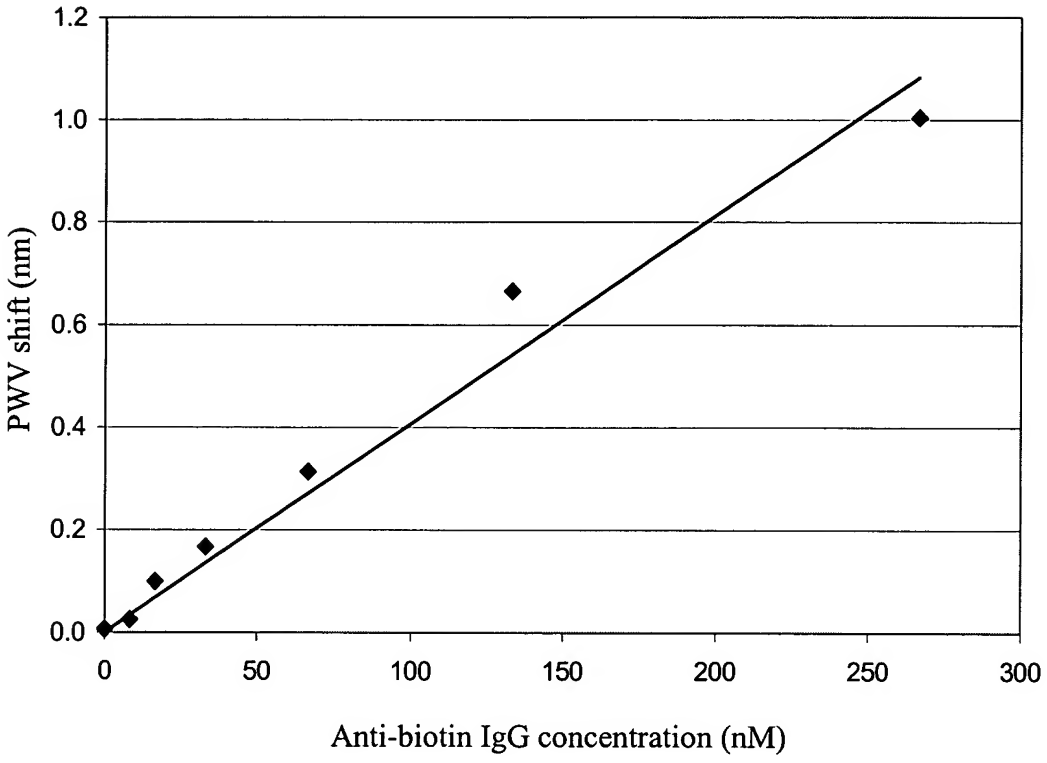
1.2
1.0
0.8
0.6
0.4
0.2
0.0
PWV shift (nm)
0
50
100
150
200
250
300
Anti-biotin IgG concentration (nM)

Figure 27

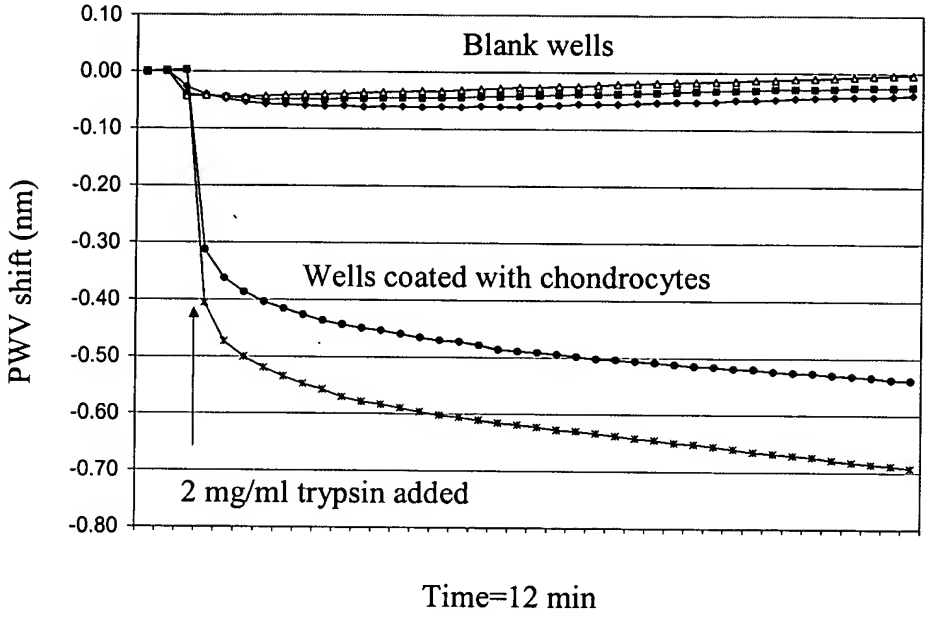
0.10
0.00
-0.10
-0.20
-0.30
-0.40
-0.50
-0.60
-0.70
-0.80
PWV shift (nm)
Blank wells
Wells coated with chondrocytes
2 mg/ml trypsin added
Time=12 min

Figure 28

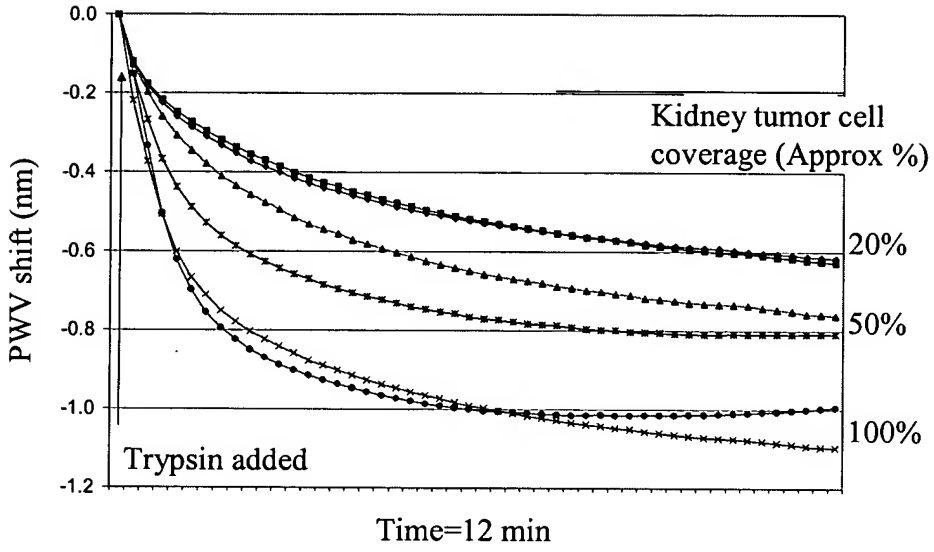
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
PWV shift (nm)
Kidney tumor cell coverage (Approx %)
20%
50%
100%
Trypsin added
Time=12 min

Figure 29

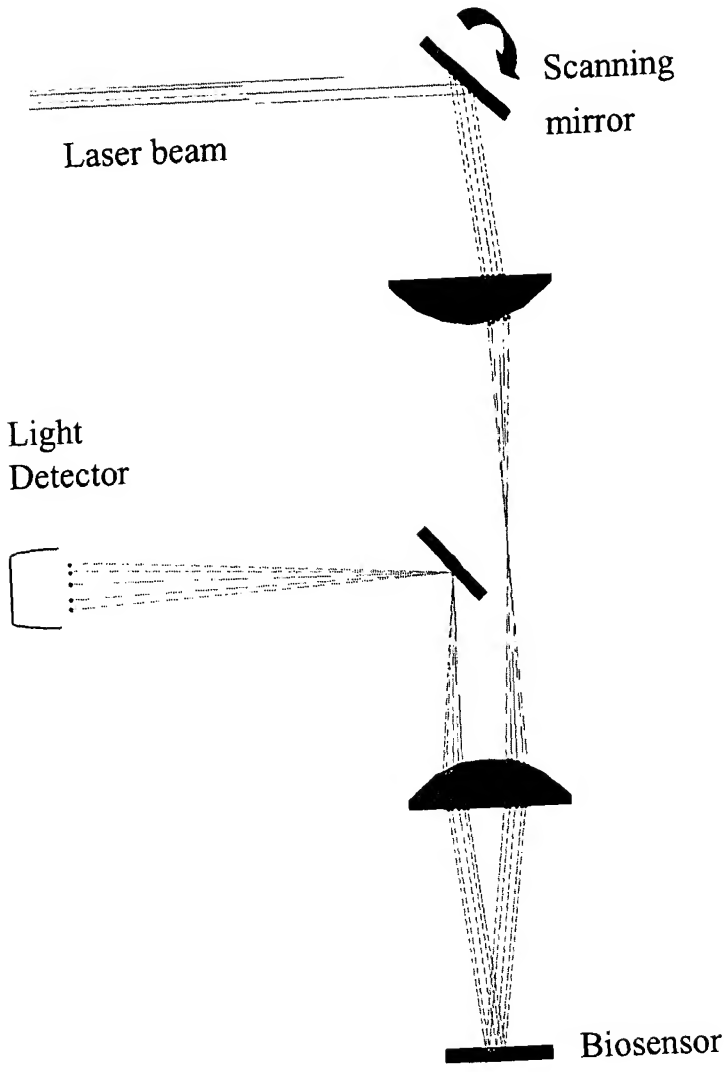

Figure 30

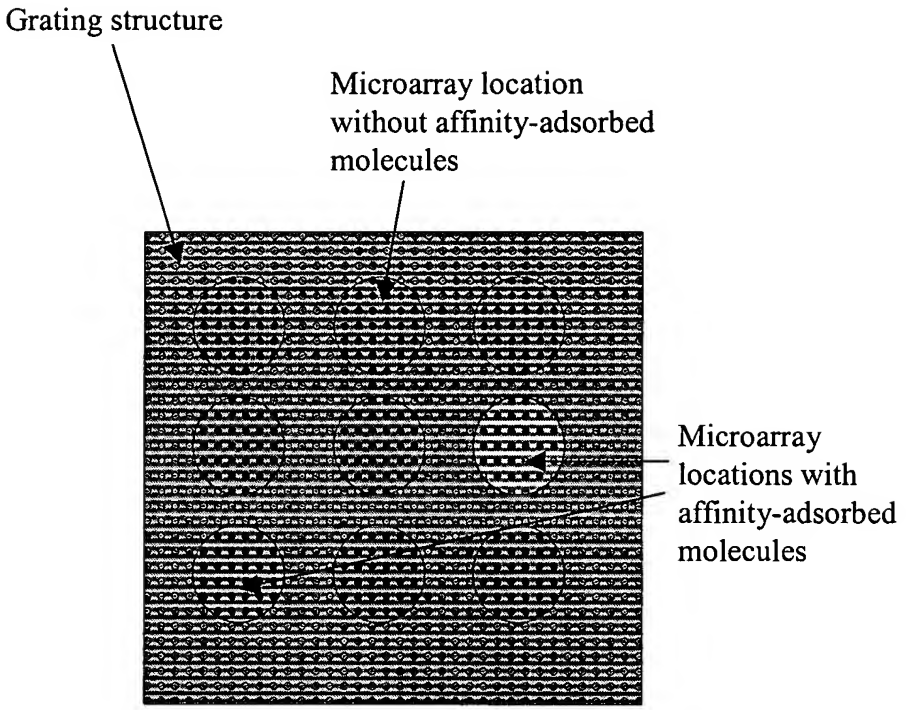
Grating structure
Microarray location without affinity-adsorbed molecules
Microarray locations with affinity-adsorbed molecules

Figure 31

- Microtiter plate

Plastic bottomless microtiter plate.
Holes in plate are open from top to bottom

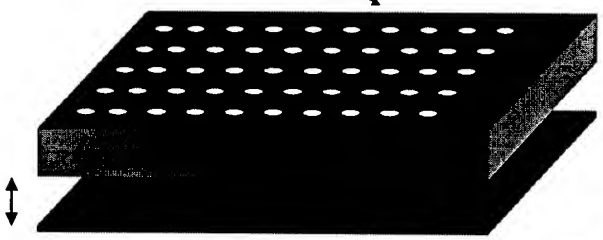

Figure 32A

- Microarray slide

Resonant reflection biosensor surface

Figure 32B

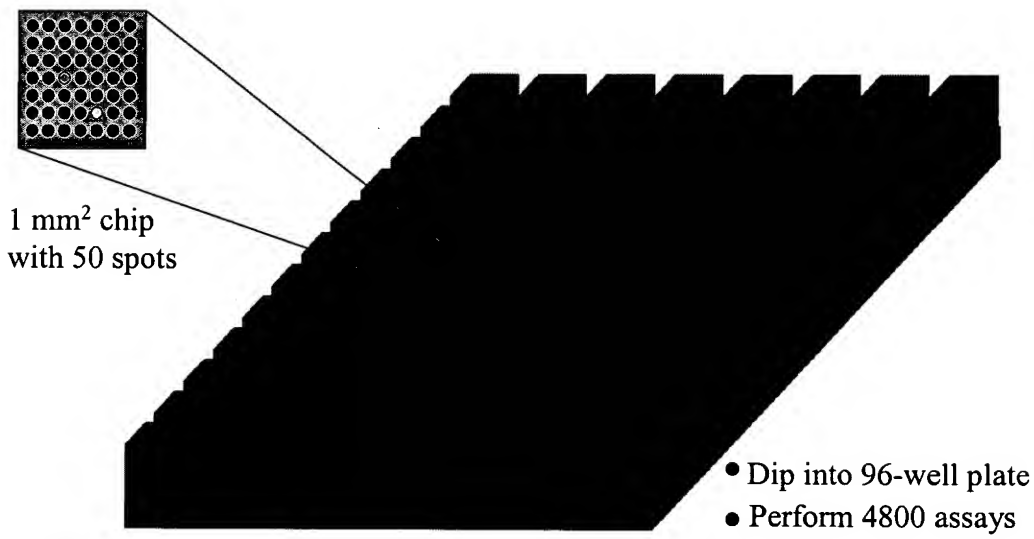
1 mm2 chip
with 50 spots
• Dip into 96-well plate
• Perform 4800 assays

Figure 33

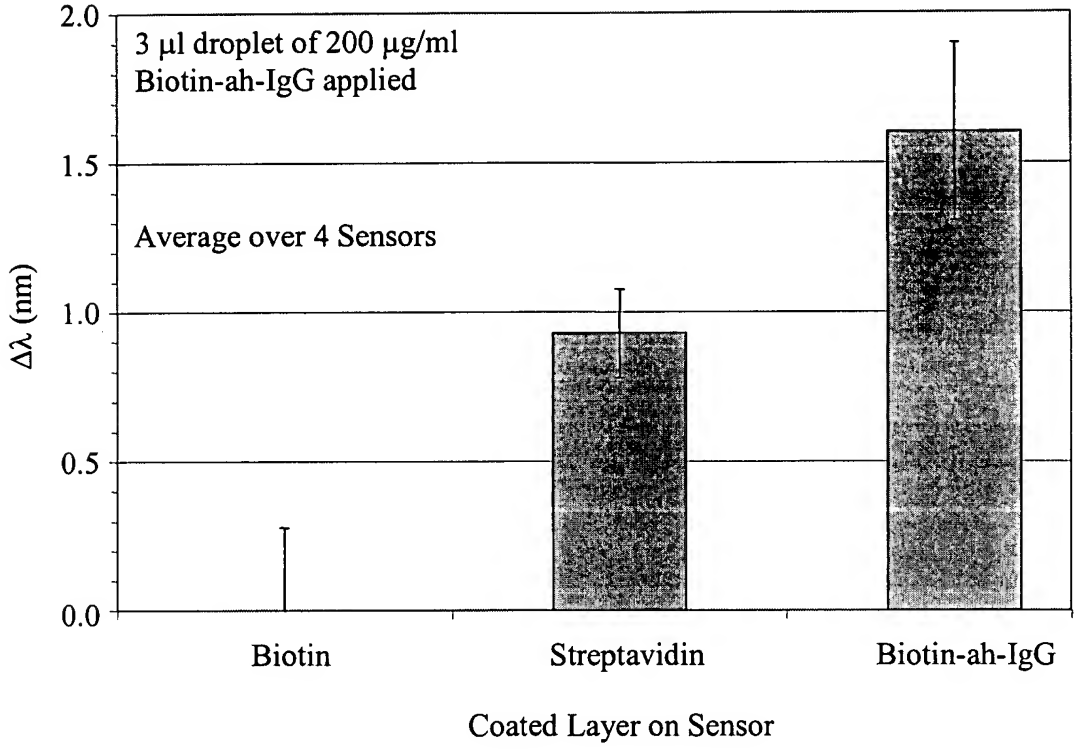

Figure 34A

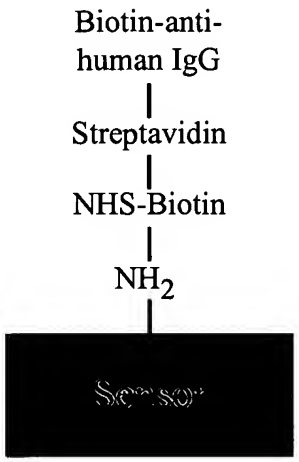

Figure 34B

SPOTTED ARRAY

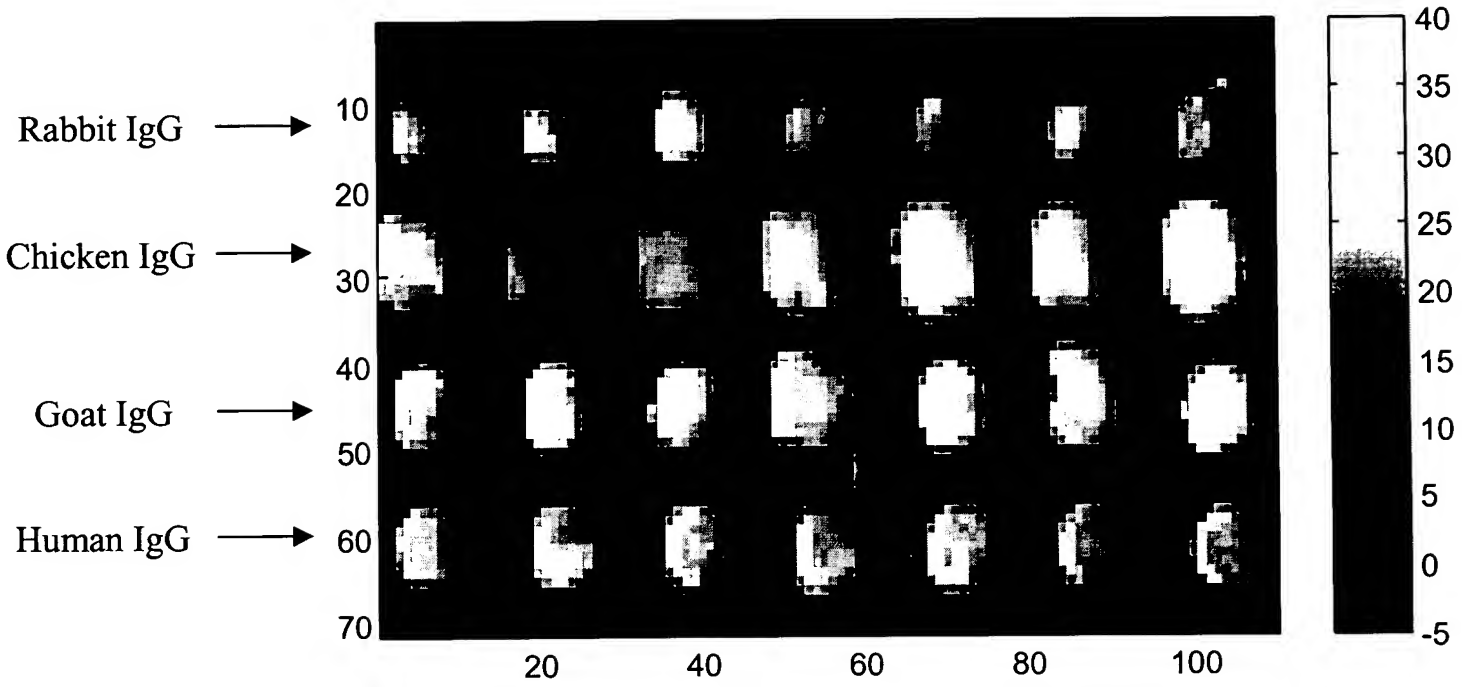

Figure 35A

BINDING (α-h-IgG) ARRAY

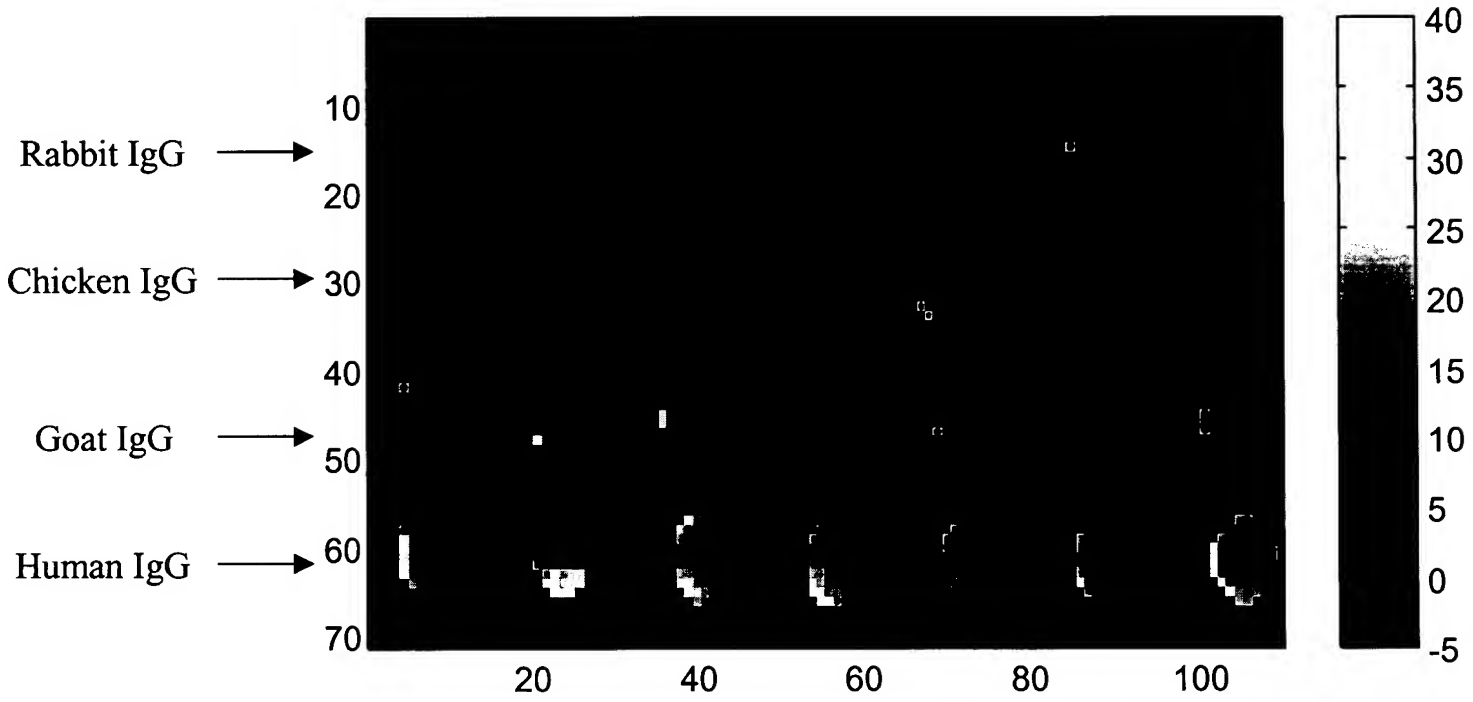

Figure 35B

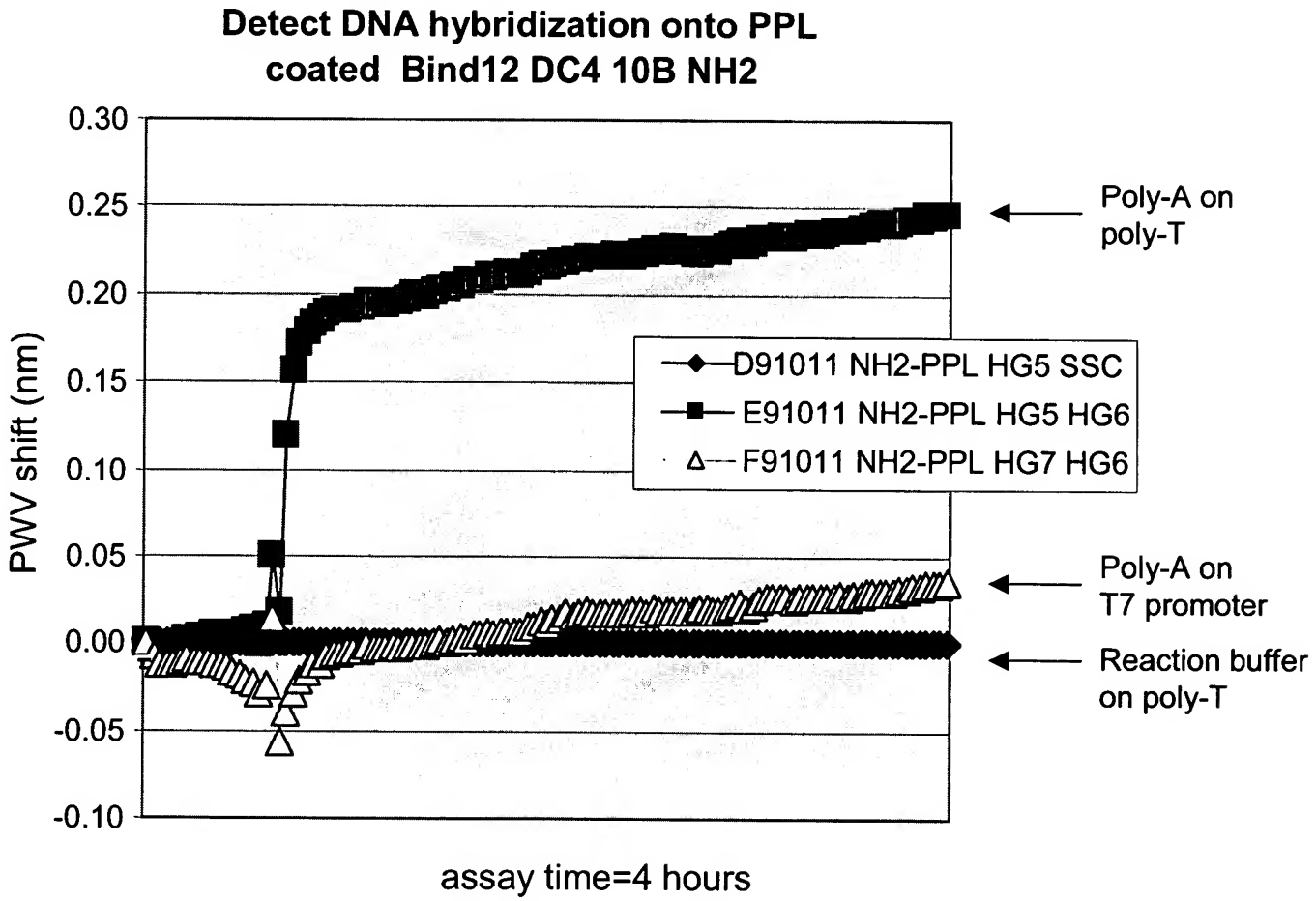
Detect DNA hybridization onto PPL coated Bind12 DC4 10B NH2
PWV shift (nm)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
assay time=4 hours
D91011 NH2-PPL HG5 SSC
E91011 NH2-PPL HG5 HG6
F91011 NH2-PPL HG7 HG6
Poly-A on poly-T
Poly-A on T7 promoter
Reaction buffer on poly-T

Figure 36A

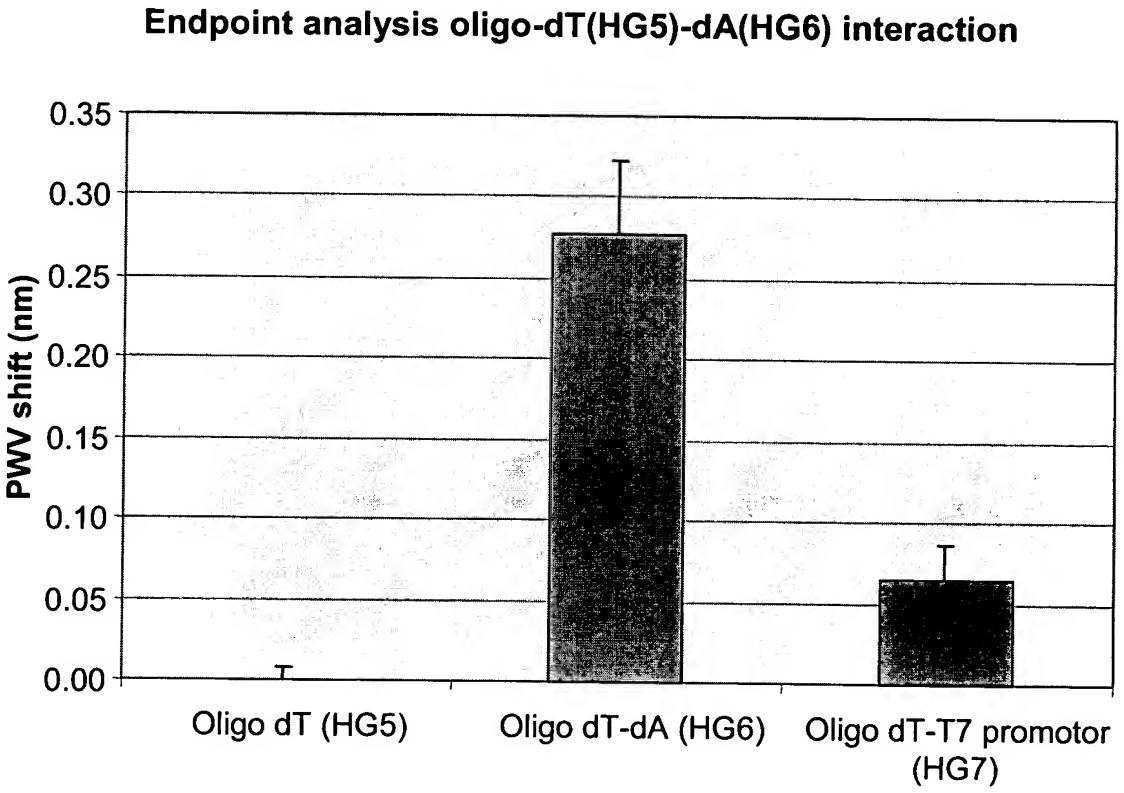
Endpoint analysis oligo-dT(HG5)-dA(HG6) interaction
PWV shift (nm)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Oligo dT (HG5)
Oligo dT-dA (HG6)
Oligo dT-T7 promotor (HG7)

Figure 36B

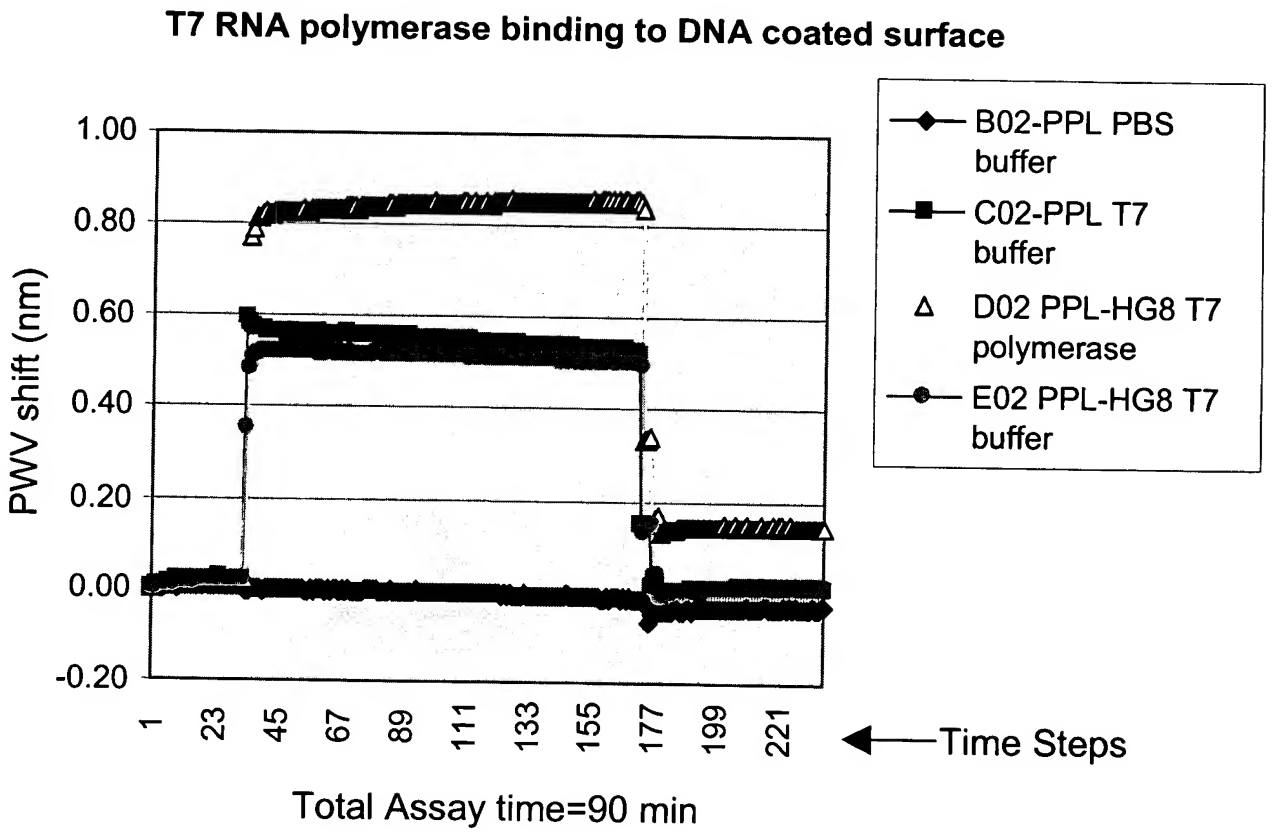
T7 RNA polymerase binding to DNA coated surface
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
PWV shift (nm)
1
23
45
67
89
111
133
155
177
199
221
Time Steps
Total Assay time=90 min
B02-PPL PBS buffer
C02-PPL T7 buffer
D02 PPL-HG8 T7 polymerase
E02 PPL-HG8 T7 buffer

Figure 37

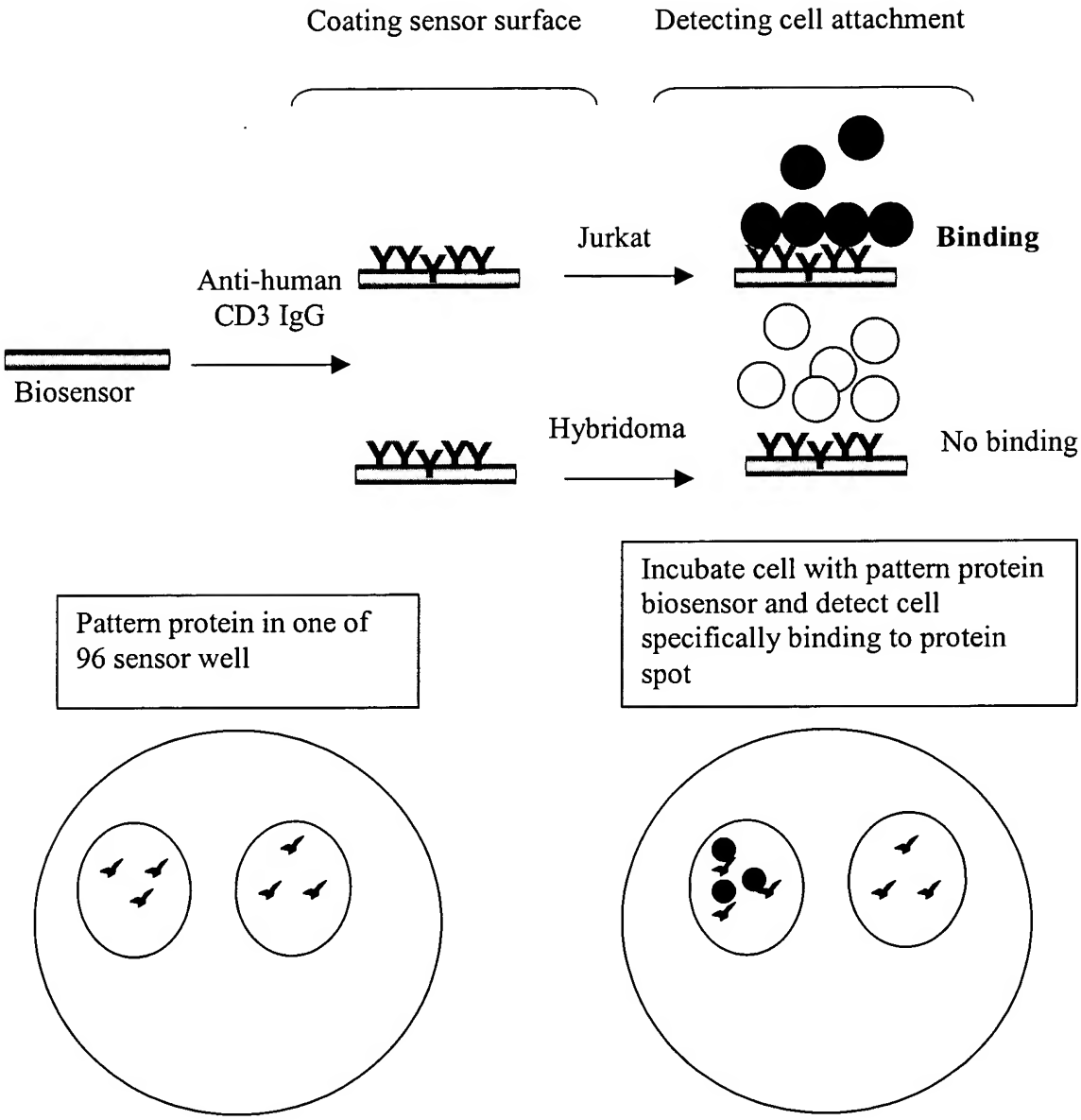
Coating sensor surface
Detecting cell attachment
Anti-human
CD3 IgG
Biosensor
Jurkat
Binding
Hybridoma
No binding
Pattern protein in one of
96 sensor well
Incubate cell with pattern protein
biosensor and detect cell
specifically binding to protein
spot

Figure 38

Biosensor image to visualize the protein coating pattern and cell attachment

PWV shifting plot as scanning cross the 6 mm in the diameter of well to analyze protein coating pattern and specific cell attachment

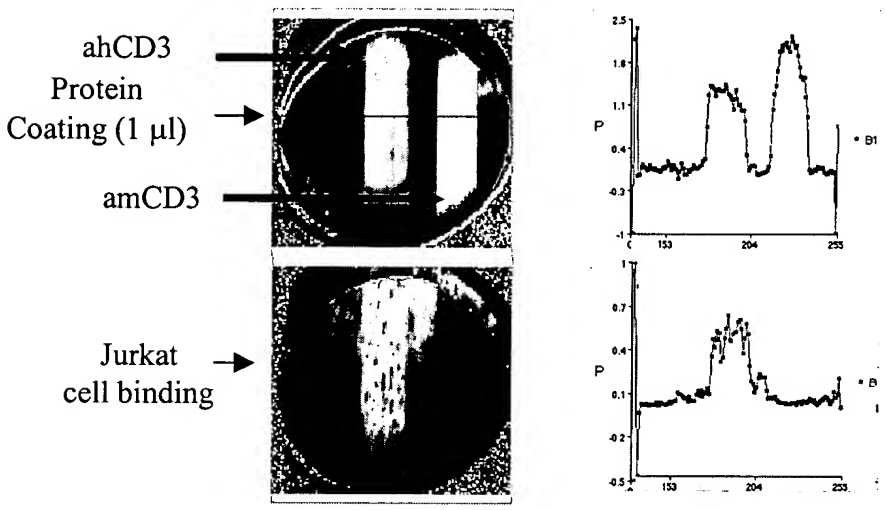

Figure 39